



1600

ENTERED

RAW SEQUENCE LISTING

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:30

Input Set : A:\umo1531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

3 <110> APPLICANT: Hale, Calvin C
 4 Price, Elmer M
 6 <120> TITLE OF INVENTION: LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT
 PROTEINS

8 <130> FILE REFERENCE: UMO 1531.1
 10 <140> CURRENT APPLICATION NUMBER: US 09/901,419A
 11 <141> CURRENT FILING DATE: 2001-07-09
 13 <160> NUMBER OF SEQ ID NOS: 5
 15 <170> SOFTWARE: PatentIn version 3.1
 17 <210> SEQ ID NO: 1
 18 <211> LENGTH: 4087
 19 <212> TYPE: DNA
 20 <213> ORGANISM: Bos taurus
 22 <220> FEATURE:
 23 <221> NAME/KEY: CDS
 24 <222> LOCATION: (268)..(3180)
 25 <223> OTHER INFORMATION:
 28 <220> FEATURE:
 29 <221> NAME/KEY: sig_peptide
 30 <222> LOCATION: (268)..(363)
 31 <223> OTHER INFORMATION:

W--> 34 <220>
 35 <221> NAME/KEY: misc_feature
 36 <222> LOCATION: (3178)..()
 37 <223> OTHER INFORMATION: A Poly (H) affinity tag comprising 6 His residues have been
 inser
 38 ted at the C-Terminus end of the coding region of the protein

W--> 41 <400> 1
 42 gaattcggga gaagccatca ccccggtct tttttcacat ccagcccatg cagaccgatc 60
 44 gccagctca accagagctg cactgatct tccacactta agcaaaccac accagtgagt 120
 46 ggcgaacatc aactcgtgct tgaaaaatac caacttgag cccggtttga gaagctacat 180
 48 cagagtctcg agatgcgacg ctacaatctg cagttttcac tagcttccca gtaggttggg 240
 50 acagttggaa ctctgccatt gcccagc atg ctg cag ttc agt ctg tca ccc acc 294
 51 Met Leu Gln Phe Ser Leu Ser Pro Thr
 52 1 5
 54 ttg tcg atg gga ttt cac gtg ata gcc atg gtg gct ctc ttg ttt tcc 342
 55 Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
 56 10 15 20 25
 58 cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac 390
 59 His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
 60 30 35 40
 62 gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att 438
 63 Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
 64 45 50 55

RECEIVED
 APR 30 2003
 TECH CENTER 1600/2900

66 tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct 486

RAW SEQUENCE LISTING

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:30

Input Set : A:\umol531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

```

67 Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
68      60      65      70
70 aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc      534
71 Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
72      75      80      85
74 tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct      582
75 Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
76 90      95      100      105
78 caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag      630
79 Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
80      110      115      120
82 aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg      678
83 Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
84      125      130      135
86 gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg      726
87 Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
88      140      145      150
90 tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg      774
91 Cys Gly His Asn Phe Thr Ala Gly Asp Leu Gly Pro Ser Thr Ile Val
92      155      160      165
94 ggg agt gct gca ttc aac atg ttc atc atc att gcc ctt tgt gtg tat      822
95 Gly Ser Ala Ala Phe Asn Met Phe Ile Ile Ile Ala Leu Cys Val Tyr
96 170      175      180      185
98 gtc gtc ccg gat ggg gag aca agg aag atc aag cat ctg cgt gtg ttc      870
99 Val Val Pro Asp Gly Glu Thr Arg Lys Ile Lys His Leu Arg Val Phe
100      190      195      200
102 ttt gtg aca gca gca tgg agc atc ttt gcc tat acc tgg ctt tac atc      918
103 Phe Val Thr Ala Ala Trp Ser Ile Phe Ala Tyr Thr Trp Leu Tyr Ile
104      205      210      215
106 att ttg tct gtc agc tcc cct ggg gtc gtg gag gtc tgg gaa ggt ttg      966
107 Ile Leu Ser Val Ser Ser Pro Gly Val Val Glu Val Trp Glu Gly Leu
108      220      225      230
110 ctt act ttc ttc ttc ttc ccc atc tgc gtt gtg ttt gct tgg gtg gca      1014
111 Leu Thr Phe Phe Phe Phe Pro Ile Cys Val Val Phe Ala Trp Val Ala
112      235      240      245
114 gac agg agg ctt ctg ttt tac aag tat gtc tac aag agg tat cgg gct      1062
115 Asp Arg Arg Leu Leu Phe Tyr Lys Tyr Val Tyr Lys Arg Tyr Arg Ala
116 250      255      260      265
118 ggc aag cag agg gga atg att att gaa cac gaa gga gac agg cca tct      1110
119 Gly Lys Gln Arg Gly Met Ile Ile Glu His Glu Gly Asp Arg Pro Ser
120      270      275      280
122 tcc aag aca gaa att gaa atg gat ggg aaa gtg gtc aat tcc cat gtt      1158
123 Ser Lys Thr Glu Ile Glu Met Asp Gly Lys Val Val Asn Ser His Val
124      285      290      295
126 gac agt ttc tta gat gga gcc ctg gtt ctg gag gtt gat gag agg gac      1206
127 Asp Ser Phe Leu Asp Gly Ala Leu Val Leu Glu Val Asp Glu Arg Asp
128      300      305      310
130 caa gat gat gaa gaa gcc agg cga gaa atg gct agg att ctg aag gaa      1254
131 Gln Asp Asp Glu Glu Ala Arg Arg Glu Met Ala Arg Ile Leu Lys Glu

```

RAW SEQUENCE LISTING

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:30

Input Set : A:\umol531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

132	315	320	325	
134	ctc aag cag aag cat cca gag aag gaa ata gag caa tta ata gaa tta	1302		
135	Leu Lys Gln Lys His Pro Glu Lys Glu Ile Glu Gln Leu Ile Glu Leu			
136	330 335 340 345			
138	gcc aat tac caa gtc tta agt cag cag caa aaa agt cga gcg ttt tac	1350		
139	Ala Asn Tyr Gln Val Leu Ser Gln Gln Gln Lys Ser Arg Ala Phe Tyr			
140	350 355 360			
142	cgt att caa gct acc cgc ctg atg acc gga gca ggc aac att tta aag	1398		
143	Arg Ile Gln Ala Thr Arg Leu Met Thr Gly Ala Gly Asn Ile Leu Lys			
144	365 370 375			
146	agg cat gca gca gac caa gcc agg aaa gct gtc agc atg cat gag gtc	1446		
147	Arg His Ala Ala Asp Gln Ala Arg Lys Ala Val Ser Met His Glu Val			
148	380 385 390			
150	aac acg gaa gtg gct gaa aat gac cct gtc agt aag atc ttc ttt gaa	1494		
151	Asn Thr Glu Val Ala Glu Asn Asp Pro Val Ser Lys Ile Phe Phe Glu			
152	395 400 405			
154	caa ggg aca tat cag tgt ctg gag aac tgt ggc aca gta gcc ctg acc	1542		
155	Gln Gly Thr Tyr Gln Cys Leu Glu Asn Cys Gly Thr Val Ala Leu Thr			
156	410 415 420 425			
158	att atc cgc aga ggt ggt gat ttg acc aac act gtg ttt gtt gac ttc	1590		
159	Ile Ile Arg Arg Gly Asp Leu Thr Asn Thr Val Phe Val Asp Phe			
160	430 435 440			
162	aga aca gag gat ggc aca gcc aat gct gga tct gat tac gaa ttt acc	1638		
163	Arg Thr Glu Asp Gly Thr Ala Asn Ala Gly Ser Asp Tyr Glu Phe Thr			
164	445 450 455			
166	gaa gga act gtg gtc ttt aag cct ggt gag acc cag aag gaa atc aga	1686		
167	Glu Gly Thr Val Val Phe Lys Pro Gly Glu Thr Gln Lys Glu Ile Arg			
168	460 465 470			
170	gtt ggc atc att gat gat gac atc ttt gag gag gat gag aat ttc ctt	1734		
171	Val Gly Ile Ile Asp Asp Asp Ile Phe Glu Glu Asp Glu Asn Phe Leu			
172	475 480 485			
174	gtg cat ctc agc aac gtc aaa gta tct ttg gaa gcc tcg gaa gac ggc	1782		
175	Val His Leu Ser Asn Val Lys Val Ser Leu Glu Ala Ser Glu Asp Gly			
176	490 495 500 505			
178	atc ctg gaa gcc agt cat gtc tct acc ctt gct tgc ctg gga tcc ccc	1830		
179	Ile Leu Glu Ala Ser His Val Ser Thr Leu Ala Cys Leu Gly Ser Pro			
180	510 515 520			
182	tcc act gcc acc gtg act att ttt gat gat gac cat gct ggc atc ttt	1878		
183	Ser Thr Ala Thr Val Thr Ile Phe Asp Asp Asp His Ala Gly Ile Phe			
184	525 530 535			
186	act ttt gag gaa ccg gtg act cat gtg agt gag agc att ggc atc atg	1926		
187	Thr Phe Glu Glu Pro Val Thr His Val Ser Glu Ser Ile Gly Ile Met			
188	540 545 550			
190	gag gtg aaa gtt ctg aga aca tct gga gca cgt gga aat gtt atc gtt	1974		
191	Glu Val Lys Val Leu Arg Thr Ser Gly Ala Arg Gly Asn Val Ile Val			
192	555 560 565			
194	ccc tat aag acc att gag ggg acc gcc aga ggt gga ggg gag gac ttt	2022		
195	Pro Tyr Lys Thr Ile Glu Gly Thr Ala Arg Gly Gly Gly Glu Asp Phe			
196	570 575 580 585			

RAW SEQUENCE LISTING

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:30

Input Set : A:\umo1531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

198	gag	gac	aca	tgc	gga	gag	ctc	gag	ttc	cag	aat	gac	gaa	att	gtc	aaa	2070
199	Glu	Asp	Thr	Cys	Gly	Glu	Leu	Glu	Phe	Gln	Asn	Asp	Glu	Ile	Val	Lys	
200				590						595					600		
202	aca	ata	tca	gtc	aag	gta	att	gat	gat	gag	gag	tat	gag	aaa	aac	aag	2118
203	Thr	Ile	Ser	Val	Lys	Val	Ile	Asp	Asp	Glu	Glu	Tyr	Glu	Lys	Asn	Lys	
204				605					610						615		
206	acc	ttc	ttc	ctt	gag	att	gga	gag	ccc	cgc	ctg	gtg	gag	atg	agt	gag	2166
207	Thr	Phe	Phe	Leu	Glu	Ile	Gly	Glu	Pro	Arg	Leu	Val	Glu	Met	Ser	Glu	
208			620					625						630			
210	aag	aaa	gcc	ctg	tta	ttg	aat	gag	ctt	ggt	ggc	ttc	aca	ata	aca	ggg	2214
211	Lys	Lys	Ala	Leu	Leu	Leu	Asn	Glu	Leu	Gly	Gly	Phe	Thr	Ile	Thr	Gly	
212		635					640					645					
214	aaa	tac	ctg	tat	ggc	cag	cct	gtc	ttc	agg	aaa	ggt	cat	gct	aga	gaa	2262
215	Lys	Tyr	Leu	Tyr	Gly	Gln	Pro	Val	Phe	Arg	Lys	Val	His	Ala	Arg	Glu	
216	650					655				660					665		
218	cat	cca	ctc	ccc	tct	act	ata	atc	acc	atc	gca	gat	gaa	tat	gat	gac	2310
219	His	Pro	Leu	Pro	Ser	Thr	Ile	Ile	Thr	Ile	Ala	Asp	Glu	Tyr	Asp	Asp	
220				670						675					680		
222	aag	cag	cca	ctg	acc	agc	aaa	gag	gag	gaa	gag	agg	cgc	att	gcg	gaa	2358
223	Lys	Gln	Pro	Leu	Thr	Ser	Lys	Glu	Glu	Glu	Glu	Arg	Arg	Ile	Ala	Glu	
224			685						690						695		
226	atg	ggg	cgc	ccc	att	ctg	gga	gag	cac	acc	aga	ctg	gag	gtg	atc	att	2406
227	Met	Gly	Arg	Pro	Ile	Leu	Gly	Glu	His	Thr	Arg	Leu	Glu	Val	Ile	Ile	
228			700					705						710			
230	gaa	gaa	tcc	tac	gag	ttc	aag	agt	acc	gtg	gac	aaa	ctg	att	aag	aag	2454
231	Glu	Glu	Ser	Tyr	Glu	Phe	Lys	Ser	Thr	Val	Asp	Lys	Leu	Ile	Lys	Lys	
232		715					720					725					
234	aca	aac	cta	gcc	ctc	gtg	ggt	ggg	acg	aac	agc	tgg	aga	gag	cag	ttc	2502
235	Thr	Asn	Leu	Ala	Leu	Val	Val	Gly	Thr	Asn	Ser	Trp	Arg	Glu	Gln	Phe	
236	730					735					740				745		
238	atc	gag	gcg	atc	act	gtc	agt	gct	ggg	gaa	gat	gac	gat	gac	gac	gaa	2550
239	Ile	Glu	Ala	Ile	Thr	Val	Ser	Ala	Gly	Glu	Asp	Asp	Asp	Asp	Asp	Glu	
240				750						755					760		
242	tgt	ggg	gag	gag	aag	ctg	ccc	tcc	tgt	ttt	gac	tac	gtg	atg	cac	ttt	2598
243	Cys	Gly	Glu	Glu	Lys	Leu	Pro	Ser	Cys	Phe	Asp	Tyr	Val	Met	His	Phe	
244			765						770						775		
246	ctg	act	gtg	ttc	tgg	aag	gtc	ctc	ttc	gcc	ttt	gtc	ccc	ccg	aca	gag	2646
247	Leu	Thr	Val	Phe	Trp	Lys	Val	Leu	Phe	Ala	Phe	Val	Pro	Pro	Thr	Glu	
248			780					785						790			
250	tac	tgg	aac	ggc	tgg	gcg	tgt	ttc	atc	gtc	tcc	atc	ctc	atg	atc	ggc	2694
251	Tyr	Trp	Asn	Gly	Trp	Ala	Cys	Phe	Ile	Val	Ser	Ile	Leu	Met	Ile	Gly	
252		795					800					805					
254	cta	ctg	acg	gct	ttc	att	gga	gac	ctc	gct	tcc	cac	ttc	gcc	tgc	acc	2742
255	Leu	Leu	Thr	Ala	Phe	Ile	Gly	Asp	Leu	Ala	Ser	His	Phe	Ala	Cys	Thr	
256	810					815					820				825		
258	atc	gcc	ctg	aag	gat	tcc	gtg	acc	gcg	gtg	gtg	ttc	ggt	gcg	ctt	gga	2790
259	Ile	Ala	Leu	Lys	Asp	Ser	Val	Thr	Ala	Val	Val	Phe	Val	Ala	Leu	Gly	
260				830						835					840		
262	acc	tca	gtg	cca	gac	aca	ttt	gca	agc	aaa	gtg	gcc	gcc	acc	cag	gac	2838

RAW SEQUENCE LISTING

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:30

Input Set : A:\umol531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

```

263 Thr Ser Val Pro Asp Thr Phe Ala Ser Lys Val Ala Ala Thr Gln Asp
264      845      850      855
266 cag tat gcg gat gca tcc ata ggt aac gtc aca ggc agc aac gcg gtg      2886
267 Gln Tyr Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val
268      860      865      870
270 aac gtc ttc ctg ggc atc ggt gtg gcc tgg tcc atc gcc gcc atc tac      2934
271 Asn Val Phe Leu Gly Ile Gly Val Ala Trp Ser Ile Ala Ala Ile Tyr
272      875      880      885
274 cac gcg gcc aac ggg gaa cag ttc aaa gtg tcc cct ggc acg cta gct      2982
275 His Ala Ala Asn Gly Glu Gln Phe Lys Val Ser Pro Gly Thr Leu Ala
276 890      895      900      905
278 ttt tct gtc act ctc ttc acc att ttt gct ttc atc aat gtg ggg gtg      3030
279 Phe Ser Val Thr Leu Phe Thr Ile Phe Ala Phe Ile Asn Val Gly Val
280      910      915      920
282 ctg ctg tat cgg cgg agg cca gaa att gga ggt gag ctg ggt ggg ccc      3078
283 Leu Leu Tyr Arg Arg Arg Pro Glu Ile Gly Gly Glu Leu Gly Gly Pro
284      925      930      935
286 cgg act gcc aag ctc ctc aca tcc tgc ctc ttt gtg ctc ctg tgg ctc      3126
287 Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp Leu
288      940      945      950
290 ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc      3174
291 Leu Tyr Ile Phe Phe Ser Ser Leu Glu Ala Tyr Cys His Ile Lys Gly
292      955      960      965
294 ttc taa aggaacaatc agatgtagta aatttatata tatatacata tatatatata      3230
295 Phe
296 970
298 cataaaaatt atgtataatg aacagaggaa actggcattt gtcattgtcca cccacctgct      3290
300 gatggaatcc agcttcaaga gcagactctg tactagggcc ggagagagaa ggcattcacct      3350
302 cccgtttccc aggggcgttc gtcttgttga accaggcatg gaggcagggc catctttacg      3410
304 tcagctcagc ccagaagcgg tgtgttctcc ccgggttcat aaatccttaa gttctttgat      3470
306 ttgttttctg tttttgcttg ttttgggtcg gggtagggag gtggttgatg ttaggggttg      3530
308 gttttggtt tgcaggggga agatcagggt ttgtggtcct cttgtgggag gtgatgtcca      3590
310 atctcaatgg taaaaatgga aatcaggaag atgactctcc ctttgcccaa aaactttaaa      3650
312 aattattttg gagtaagaaa ggaaacgggc atggaagaag aaagaagcat gtcttcacca      3710
314 tattactaaa tttcatgcct tatctctgga gtgggagcag aggtgaagtc ctccctccaa      3770
316 gaagaaacag gggagctgga atggagccaa gaagagtcac ggttctagat acagtctgat      3830
318 gtttaaagat acatcgctgc ctggcaccct tgttcaacag gtacaaaaac aacatgccta      3890
320 gattcccagg aacgcacaaa gtcctttctt atctcttcag cgctggactg tgattagcaa      3950
322 ggccctgatt ctgatgttct acacccgctg attccccagc cctcccatcc caaaccctt      4010
324 ctccggaccc ttaccctc gtacaaacag gaagaataac tccattcaaa aagcacacca      4070
326 tcctttccat tcgcatc      4087
329 <210> SEQ ID NO: 2
330 <211> LENGTH: 970
331 <212> TYPE: PRT
332 <213> ORGANISM: Bos taurus
334 <220> FEATURE:
335 <221> NAME/KEY: misc_feature
336 <222> LOCATION: (3178)..()
337 <223> OTHER INFORMATION: A Poly (H) affinity tag comprising 6 His residues have been

```

inser

VERIFICATION SUMMARY

DATE: 04/24/2003

PATENT APPLICATION: US/09/901,419A

TIME: 16:23:31

Input Set : A:\umo1531.txt

Output Set: N:\CRF4\04242003\I901419A.raw

L:34 M:258 W: Mandatory Feature missing, <223> Blank for SEQ#:1,Line#:25

L:41 M:258 W: Mandatory Feature missing, <223> Blank for SEQ#:1,Line#:31